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Ye. D. Val'ter

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On the participation of isopods in the life cycle of *Contracaecum aduncum* (Ascaridata, Anisakoidea)

by Ye.D. Val'ter

Department of Invertebrate Zoology of Moscow State University

Our experiment to infect isopods *Iaera albifrons ischiosetosa* with *Contracaecum aduncum* larvae produced positive results. In this experiment, we traced some of the features of the organogenesis of the *C. aduncum* larvae (development of the digestive and reproductive systems). The spontaneous infection of the isopods with this parasite confirms the possibility of their participation as an intermediate host in the life cycle of the fish parasite *C. aduncum*.

Planktonic and benthic crustaceans play no small role in the life cycle of the nematode *Contracaecum aduncum* (Rudolphi, 1802) Baylis, 1920. Markowski (1937) has experimentally proven that copepods of the species *Acartia bifilosa* and *Eurytemora affinis* contribute to the life cycle of *Contracaecum aduncum*. Punt (1941), who has also studied the life cycle of this parasite, placed *C. aduncum* larvae in an extract from planktonic animals without result; a positive result was obtained when the river crustacean *Potamobius astacus* was infected. Margolis and Butler (1954) demonstrated a high degree of infestation of the pink shrimp *Pandalus borealis* with mature and immature nematodes of the species *Contracaecum aduncum*.

We (Popova and Val'ter, 1965) have experimentally proven that eight species of Copepoda participate in the life cycle of *C. aduncum*. The most extensive and intensive infestation is noted in *Temora longicornis*, *Pseudocalanus elongatus* and *Acartia longiremis*. Highly extensive infestation (8.6%) was noted when White Sea *Caprella septentrionalis* were analyzed for spontaneous infection with *C. aduncum* larvae (Val'ter, 1968). The present paper discusses how the life cycle of *C. aduncum* is promoted by yet another representative of benthic

crustaceans, *Iaera albifrons ischiosetosa* Forsman, 1949.[1] We do not know of any other data on the parasitization of *I. albifrons* by the larvae of *C. aduncum* and other nematodes. Having studied the biology of *I. albifrons*, Forsman (1944) discovered trematode cysts in the body cavity of these isopods. In Uspensky's work devoted to the parasitic fauna of benthic crustaceans of the Barents Sea (Uspensky, 1963), *Iaera albifrons* is given as the primary intermediate host of the trematode *Podocotyle atomon*.

In the process of studying the biology of the fish parasite *C. aduncum* at the White Sea Biological Station of Moscow University in 1966, we performed an experiment in which we infected isopods *Iaera albifrons ischiosetosa*, collected at the mouth of Bannyi Creek during low tide, with the larvae of this parasite. Into glass crystallizers measuring 30 cm in diameter and half-filled with seawater, we placed a culture of 13-day-old *C. aduncum* larvae at the 2nd stage of development, which congregated at the bottom. The largest and most active isopods were then placed into these crystallizers. Small stones and fragments of wood from the place where the isopods were collected served as the substrate. Small bits of sea algae (*Ascophyllum*) served as an additional substrate and food for the crustaceans. After a period of two days, i.e. sufficient time, in our opinion, for the larvae to infect the crustaceans, the crystallizers were placed in a channel to create the best possible conditions for the isopods. During the winter, fresh seawater was added to the crystallizers once every ten days and the algae were changed once a month because the channel was not operational during this period. The experimental isopods were placed under close observation. At certain intervals, the crustacens were dissected, primarily the weakened ones.

The isopods were fixed in Barbagallo fluid. The *C. aduncum* larvae found in them were placed in a weak solution of glycerin with water or lactic acid for clarification. A weak (0.25%) solution of toluene blue was sometimes used to bring out this or that system of organs in the parasite. The experiment was carried out twice, and both times the result was positive. The isopods in the control crystallizers remained

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[1]We are greatly indebted to Yu.M. Frolov for his extensive assistance in identifying the isopods.

uninfected. The larvae used to infect the isopods measured 0.328-0.420 mm in length with the larva case, and 0.254-0.368 mm without it; the maximum width of the larva case was 0.038-0.044 mm, and the width of the body was 0.013-0.016 mm. The results of the experimental infection of the isopods are given in table 1.

Table 1. Experimental infection of *Iaera albifrons ischiosetosa* with *Contracaecum aduncum* larvae

Exp. No.	Beginning of experiment	Length of experiment (days)	No. of isopods in experiment	Extensiveness of infection, %	Intensity of infection
1	31 Aug 1966	54	30	70.6	2-14
2	9 Sept 1966	207	43	92.3	1-11

The infection of the isopods with *C. aduncum* larvae enabled us to observe the growth and development of the parasite over a period of seven months.

Due to the fact that the experimental isopods were not transferred from the crystallizers to a larva-free environment after a sufficient infection period, larvae of different instars were found in them upon dissection at different intervals. Considering the fact that *C. aduncum* larvae can live in seawater for about two months, it becomes obvious why infection of the isopods had to be repeated. However, we had no intention of tracing the entire ontogenesis of the larvae inside the isopods. Our purpose was to establish whether the latter could become infected, and to study some of the features of organogenesis in the larvae of the parasite.

The parasitic effect of a large number of larvae was bound to show on the condition of the ispods. As the larvae grew, they suppressed the development of the isopods, and the latter died. Only those infested by a relatively small number of larvae (1-3) survived.

As our investigations have shown, the *C. aduncum* larvae become localized in the body cavity of the isopod, concentrating in the area of the intestinal tube and gonads. With a high intensity of infection (10-14 larvae), they are distributed more or less uniformly throughout the hemocoel, sometimes finding their way into the leg muscles. One larva

was found in the 2nd segment (basipodite) of the right gressorial leg; the anterior one-third of the larva had pierced the leg, while the rest of it projected freely into the body cavity of the isopod.

On the second day after infection, *C. aduncum* which had shed the larva case (0.256-0.344 mm long) were found in the body cavity of the isopods; a piercing tooth was clearly discernible at the anterior end of the body in the larvae. The digestive system had the form of a tube which was clearly differentiated into an oesophagus, a ventriculus, and an intestine with yellowish granular contents. Larvae measuring 0.323-0.373 mm in length, observed on the 3rd-7th day of the experiment, did not differ in any way from the larvae described above. On the 27-30th day after infection of the isopods, the larvae had hardly increased in length (table 2). They were characterized by a caecum extending from the ventriculus, but there was still no intestinal caecum. The lips were already forming. In isopods dissected on the 42nd day after infection, the larvae were very thick (width of body 0.025 mm), and they measured 0.344-0.441 mm in length. There was still no intestinal caecum.

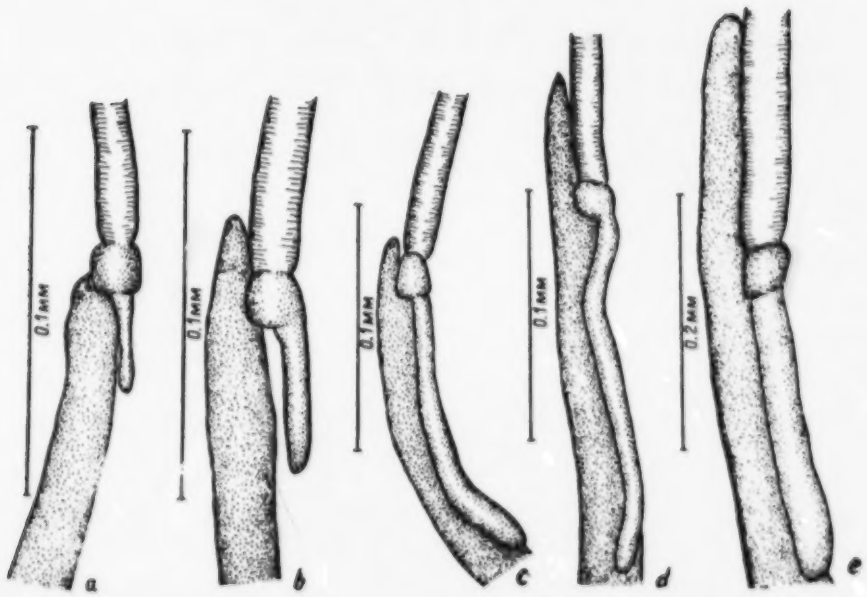

Fig. 1. a-e - development of gastric and intestinal caeca in 3rd-instar larvae of *Contracaecum aduncum* from *Iaera albifrons isciosetosa*

We did not manage to observe the molting of the *C. aduncum* larvae. Judging by their morphology, we presume that the molting took place between the 40-50th day. Therefore, the larvae up to the 40-50th day after infection were regarded as 3rd-instar larvae; from the 40-50th day up to the end of the experiment, we encountered larvae of different size and, therefore, different age, which belonged to the 2nd stage of development. On the 54th day, dissected isopods were found to contain parasites ranging from 0.418 to 0.677 mm in length. All of them had an intestinal caecum. In the smallest one, it barely protruded, comprising approximately one-half of the length of the ventriculus (Fig. 1, a); in others, it was already overlapping the anterior margin of the ventriculus (Fig. 1, b), and in some it constituted about one-half of the length of the gastric caecum. The gastric caecum also varied in its development, but it was always longer than the intestinal one (Fig. 1, c, d). By this time, the larvae already had a discernible genital rudiment 0.020-0.032 mm long and 0.011-0.014 mm wide at about the middle of the body (Fig. 2, a, e). On the 84th day of the experiment, the digestive system was already fully formed in 2.030-2.088 mm larvae. The length of the gastric and intestinal caeca was nearly the same (0.222-0.265 and 0.208-0.276 mm respectively) (Fig. 1, e). There were no changes in the reproductive system.

On the 108th day after infection, the *C. aduncum* larvae extracted from the isopods varied in size (1.261-4.824 mm long). In all of the larvae measured, with the exception of one, the gastric caecum was longer than the intestinal one. The larvae could already be differentiated by sex (Fig. 2, b, c, f). In the male larvae, the genital rudiment measured 0.029-0.043 mm in length and 0.019-0.21 mm in width; a genital tube 0.087-0.305 mm long and 0.010-0.031 mm wide extended from it posteriorly, and a genital tube 0.031 mm long and 0.016 mm wide extended anteriorly; the distance from the beginning of the genital tubes to the anterior end of the body of the parasite measured 1.066-1.866 mm. In the female, the uterine body measured 0.016 mm in length and 0.023 mm in width, the right horn 0.026 mm in length and 0.0087 mm in width, and the left horn 0.032 mm in length and 0.0073 mm in width. However, not all the elements of the reproductive system were clearly defined in this larva at this point. In another female larva, the

reproductive system had undergone greater differentiation; from a genital rudiment (uterine body) 0.042 mm long and 0.016 mm wide, two uterine horns extended posteriorly, the right one measuring 0.3906 mm long and 0.035 mm wide, and the left one 0.251 mm long and 0.011 mm wide; a thin tubule (vagina) measuring 0.062 mm in length and 0.016 mm in width extended anteriorly (Fig. 2, d).

When isopods were dissected on the 116th, 123rd and 148th day, the larvae of *C. aduncum* hardly differed in size from the ones described above (body length 1.720-3.386 mm) (table 2). In one of them (would-be male) which measured 3.277 mm in length, the reproductive system consisted of a tube 0.769 mm long and 0.012 mm wide. The distance from its beginning to the anterior end measured 1.827 mm, and the distance from the same point to the posterior end was 1.450 mm. The larvae in the isopods reached a length of 5.472 mm on the 175th day, and 7.229 mm on the 207th day. As the parasite grew in size, its organs continued to grow and develop. On the 207th day of the experiment, a male larva had a genital tube measuring 2.966 mm in length and 0.043 mm in width. Thus, the reproductive system in older larvae occupied nearly one-third of the total length of the body. As a rule, the gastric caecum was longer than the intestinal one in larvae of different instars; we found that the intestinal caecum was longer than the gastric one in only 4 of the 33 larvae measured.

The substantial increase in size (table 2) and the morphological changes observed in the *C. aduncum* larvae inside the isopods permit us to assume that *laera albifrons ischiosetosa* is an intermediate host of this nematode. The likelihood of this has been confirmed by the spontaneous infestation observed by us. In 1966, 927 isopods were collected from July to September in the vicinity of the Moscow University Biological Station, on Bannyi Creek near Chernyye Shcheli, and in the vicinity of the Biological Station of the Zoological Institute (Kartesh), on a creek in Kruglaya Bay and in Medvezhya Bay; one larva of *Contracaecum* was found in one of these isopods. In 1967, 1118 isopods were collected from May to September in the vicinity of the Moscow University Biological Station, and one larva of *Contracaecum* was found in two of them.

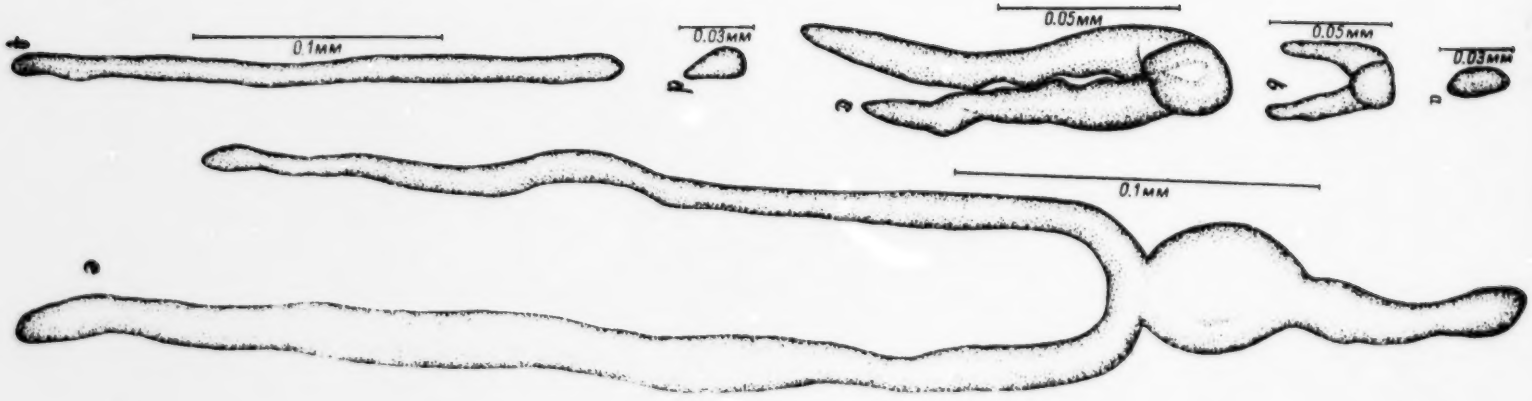

Fig. 2. Development of the reproductive system, female (a, b, c, d) and male (e, f), in 3rd-instar larvae of *Contracaecum aduncum* extracted from *laera albifrons ischiosetosa*

Table 2. Comparative dimensions (mm) of *Contracaecum aduncum* larvae of different age from the body cavity of *laera albifrons ischiosetosa*

	Day of dissection of isopods					
	1st	28th	54th	108th	175th	207th
	2nd instar		3rd instar			
Maximum length of body	0.256-0.344	0.357-0.374	0.418-0.677	1.261-4.824	3.989-5.472	7.229
Maximum width of body	0.014-0.016	0.017-0.020	0.026-0.043	0.049-0.131	0.116-0.129	0.165
Width in region of lips	-	0.010-0.015	0.014-0.020	0.017-0.043	0.040-0.050	0.057
Width in region of ventriculus	-	0.017	0.026-0.043	0.038-0.131	0.105-0.116	0.162
Width in region of anus	-	0.010-0.012	0.017-0.026	0.032-0.063	0.063-0.064	0.083
Length of oesophagus	-	-	0.105-0.168	0.227-0.704	0.640-0.850	1.037
Width of oesophagus	-	-	0.007-0.014	0.022-0.043	0.043-0.048	0.063
Length of ventriculus	-	0.017	0.011-0.017	0.014-0.034	0.040-0.063	0.086
Width of ventriculus	-	0.013	0.011-0.017	0.019-0.048	0.054-0.054	0.074
Length of gastric caecum	-	0.042	0.031-0.148	0.200-0.370	0.388-0.402	0.388
Width of gastric caecum	-	0.008	0.006-0.017	0.019-0.046	0.034-0.066	0.074
Length of intestinal caecum	-	0.057-0.059	0.006-0.063	0.099-0.342	0.345-0.495	0.214
Width of intestinal caecum	-	-	0.006-0.020	0.013-0.046	0.042	0.086
Distance from excretory pore to anterior end of body	-	-	0.091	0.110-0.217	0.245-0.285	0.326
Distance from anus to tip of tail	-	-	0.054-0.068	0.078-0.125	0.108-0.182	0.140
Distance from nerve ring to anterior end of body	-	-	0.074-0.080	0.099-0.188	0.185-0.219	0.265
Height of larval tooth	-	-	0.00285-0.00485	0.0057-0.0058	0.010-0.011	0.010
Length of caudal spine	-	-	-	0.013-0.017	0.017	-
Width of caudal spine	-	-	-	0.0036-0.0044	0.0042-0.0050	-

The parasites extracted from the isopods were quite large, and they were quite similar in structure and size to the *C. aduncum* larvae from the experimentally infected isopods. The measurements of these larvae were as follows: body length 1.914-2.871 mm, width 0.067-0.110 mm; width in the region of the lips 0.026-0.038 mm; width in the region of the ventriculus 0.067-0.110 mm; width in the region of the anus 0.035-0.058 mm; length of the ventriculus 0.017-0.029 mm, width 0.029-0.042 mm; length of intestinal caecum 0.241-0.377 mm, width 0.026-0.043 mm; length of gastric caecum 0.230-0.309 mm, width 0.020-0.026 mm; distance from the anus to the tip of the tail 0.084-0.104 mm; length of oesophagus 0.365-0.536, width 0.028-0.033 mm; distance from the excretory pore to the anterior end of the body 0.171-0.203 mm; distance from the nerve ring to the anterior end of the body 0.104-0.160 mm; length of the caudal spine 0.013 mm, width 0.004 mm.

The larvae from spontaneously infected isopods were identical to the larvae found in experimentally infected isopods on the 80-108th day (3rd-instar larvae 3-3 1/2 months old). Consequently, while *C. aduncum* larvae were detected in September in spontaneously infected isopods, the invasion of the latter by the larvae of the parasite occurred approximately in the middle of June—beginning of July.

Therefore, the fact that we observed spontaneous infection of isopods by *C. aduncum* larvae confirms that these crustaceans may participate in the life cycle of this nematode as an intermediate host.

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